

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number  
WO 01/69200 A3

(51) International Patent Classification<sup>7</sup>: C12Q 1/68

(21) International Application Number: PCT/US01/07858

(22) International Filing Date: 12 March 2001 (12.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/188,669	11 March 2000 (11.03.2000)	US
60/228,885	29 August 2000 (29.08.2000)	US
60/234,229	21 September 2000 (21.09.2000)	US
60/234,363	22 September 2000 (22.09.2000)	US
60/242,770	23 October 2000 (23.10.2000)	US
60/242,840	23 October 2000 (23.10.2000)	US

(71) Applicant: FRESHGENE, INC. [US/US]; 335 Middlefield Road, Mountain View, CA 94043 (US).

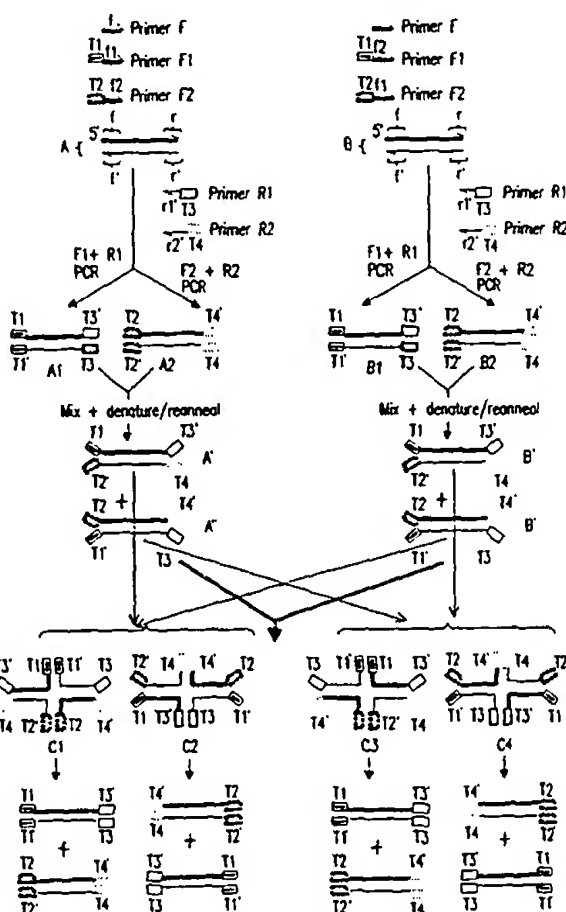
(72) Inventors: YANG, Qinghong; 1901 Rock Avenue, Apt. #108, Mountain View, CA 94043 (US); YANG, Wendy; 1901 Rock Avenue, Apt. #108, Mountain View, CA 94043 (US); LISHANSKI, Alla; 840 Meridian Way, #110, San Jose, CA 95126 (US).

(74) Agents: WEILD, David, III et al.; Pennic & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

[Continued on next page]

(54) Title: METHODS FOR DETECTION OF DIFFERENCES IN NUCLEIC ACIDS



(57) Abstract: The present invention provides methods for detecting the presence or absence of a difference between two related nucleic acid sequences. In the methods, a target nucleic acid and a reference nucleic acid are contacted under conditions in which they are capable of forming a four-way nucleic acid complex with a branch structure that is capable of migration. Under the contact conditions, if the reference nucleic acid and target nucleic acid are identical, branch migration is capable of going to completion resulting in complete strand exchange. If the reference nucleic acid and target nucleic acid are different, branch migration does not go to completion, resulting in a stable four-way complex. Detection of the stable four-way complex identifies the presence of a difference between the nucleic acids. A stable four-way complex can be detected with molecules that specifically bind such complexes, by gel electrophoresis or by specific isolation of the stable four-way complex.

WO 01/69200 A3

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US01/07858

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/68  
US CL : 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

USPAT, DERWENT WPI, MEDLINE, BIOSIS  
search terms: holliday junction, mutation, resolved, duplex, complex

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of documents, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,013,439 A (LISHANSKI et al.) 11 January 2000.	1-18
A	US 5,824,471 A (MASHAL et al.) 20 October 1998.	1-18
A	US 5,698,400 A (COTTON et al.) 16 December 1997.	1-18
A	WO 97/23646 A1 (BEHRINGWERKE AKTIENGESELLSCHAFT) 03 July 1997.	1-18

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

26 APRIL 2001

Date of mailing of the international search report

05 JUN 2001

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

KENNETH R. HORLICK

Telephone No. (703) 308-3909